

## VU Research Portal

### **Improvement of glycaemic control in type 2 diabetes: favourable changes in blood pressure, total cholesterol and triglycerides, but not in HDL cholesterol, fibrinogen, von Willebrand factor and (pro)insulin**

Becker, A.; van der Does, F.E.E.; van Hinsbergh, V.W.M.; Heine, R.J.; Bouter, L.M.; Stehouwer, C.D.A.

#### ***published in***

The Netherlands Journal of Medicine  
2003

#### ***document version***

Publisher's PDF, also known as Version of record

[Link to publication in VU Research Portal](#)

#### ***citation for published version (APA)***

Becker, A., van der Does, F. E. E., van Hinsbergh, V. W. M., Heine, R. J., Bouter, L. M., & Stehouwer, C. D. A. (2003). Improvement of glycaemic control in type 2 diabetes: favourable changes in blood pressure, total cholesterol and triglycerides, but not in HDL cholesterol, fibrinogen, von Willebrand factor and (pro)insulin. *The Netherlands Journal of Medicine*, 61(4), 129-136.

#### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

#### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

#### **E-mail address:**

[vuresearchportal.ub@vu.nl](mailto:vuresearchportal.ub@vu.nl)

# Improvement of glycaemic control in type 2 diabetes: favourable changes in blood pressure, total cholesterol and triglycerides, but not in HDL cholesterol, fibrinogen, von Willebrand factor and (pro)insulin

A. Becker<sup>1</sup>, F.E.E. van der Does<sup>2</sup>, V.W.M. van Hinsbergh<sup>3,4</sup>, R.J. Heine<sup>1,5</sup>,  
L.M. Bouter<sup>1,6</sup>, C.D.A. Stehouwer<sup>1,4,7\*</sup>

<sup>1</sup>Institute for Research in Extramural Medicine, <sup>4</sup>Institute for Cardiovascular Research, Departments of <sup>5</sup>Endocrinology, <sup>6</sup>Epidemiology and Biostatistics, and <sup>7</sup>Internal Medicine, Free University Medical Centre, De Boelelaan 1117, 1081 HV Amsterdam, the Netherlands, tel.: +31 (0)20-444 43 09, fax: +31 (0)20-444 43 13, e-mail: cda.stehouwer@vumc.nl, <sup>2</sup>General Practitioner, Rotterdam, the Netherlands, <sup>3</sup>Gaubius Laboratory, TNO Prevention and Health, Leiden, the Netherlands, \* corresponding author

## ABSTRACT

**Background:** Diabetes mellitus causes a substantial increase in cardiovascular risk, which can only partly be reduced by antihyperglycaemic treatment. We were interested in whether improvement in glycaemic control is associated with improvement of other cardiovascular risk factors. Therefore, we studied among type 2 diabetic patients the association between on the one hand changes in glycaemic control and on the other hand within-subject changes of both classic cardiovascular risk factors and less conventional cardiovascular risk indicators that are typically associated with type 2 diabetes (proinsulin, insulin, fibrinogen, von Willebrand factor and the urinary albumin-creatinine ratio).

**Methods:** The 214 type 2 diabetic patients were randomly assigned to either a strict fasting capillary glucose target level (<6.5 mmol/l) or a less strict target (<8.5 mmol/l). Duration of follow-up was two years. Since the interventions did not yield statistically significant differences between the treatment arms, we reanalysed the data focusing on within-subject changes of cardiovascular risk factors and indicators across tertiles of average HbA<sub>1c</sub>.

**Results:** Individuals in whom HbA<sub>1c</sub> decreased had significant favourable concurrent changes in triglycerides, total cholesterol, blood pressure, and in the albumin-creatinine ratio in those who were normoalbuminuric at baseline. In contrast, these individuals had unfavourable, although not statistically significant, changes in HDL cholesterol, proinsulin, insulin, fibrinogen and von Willebrand factor. In the whole group, fibrinogen increased more than could be expected on the basis of the relationship between fibrinogen and age, namely from  $3.5 \pm 0.8$  to  $3.9 \pm 0.9$  g/l (p value <0.01).

**Conclusions:** Our results suggest that improvement in glycaemia in type 2 diabetes is associated with significant favourable changes in triglycerides, total cholesterol, blood pressure and, in normoalbuminuric individuals, albumin-creatinine ratio. In contrast, it is not consistently associated with favourable changes in some cardiovascular risk indicators typically associated with diabetes, which may in part explain why antihyperglycaemic treatment does not clearly lower atherothrombotic disease risk.

## INTRODUCTION

Type 2 diabetes carries an increased risk of cardiovascular disease, which is not fully explained by several well-established risk factors (i.e., variables causally related to cardiovascular disease) associated with type 2 diabetes.<sup>1,2</sup> Hyperglycaemia, an important factor in causing microangiopathy in type 1 and type 2 diabetes,<sup>3-6</sup> is an additional possible explanation for the enhanced cardiovascular risk.<sup>7-12</sup> However, the United Kingdom Prospective Diabetes Study (UKPDS) showed that over the first ten years after diagnosis, intensive glucose-control treatment, when compared with conventional treatment, reduced the frequency of microvascular complications but not diabetes-related mortality or myocardial infarction.<sup>13</sup> Furthermore, the feasibility trial of the Veterans Affairs Cooperative Study Group (VA CSDM), which compared randomly allocated intensive and conventional glycaemic control, showed a trend towards an adverse effect of intensive glycaemic control on cardiovascular mortality.<sup>14</sup>

Another explanation for the excess cardiovascular risk in type 2 diabetes may lie in less conventional cardiovascular risk indicators (i.e., variables whose association with cardiovascular disease may or may not be causal), such as proinsulin and insulin levels, and haemostatic and fibrinolytic abnormalities, which are typically associated with type 2 diabetes. For example, high levels of fibrinogen may partially explain the excess cardiovascular risk in type 2 diabetes.<sup>15-17</sup> In addition, cardiovascular risk may be increased by high von Willebrand factor levels (vWf) and microalbuminuria,<sup>18-25</sup> which may both reflect endothelial dysfunction.<sup>19,21,26</sup>

We present data on a well-defined cohort of 214 type 2 diabetic patients followed for two years. After the baseline assessment, patients were randomly assigned to either a strict fasting capillary blood glucose target level (<6.5 mmol/l), or a less strict target (<8.5 mmol/l). This study design allowed us to study the effects of two different levels of treatment intensification and analyse the associations between within-subject changes of glycaemia, and changes in lipidaemia, blood pressure, proinsulin, insulinaemia, plasma fibrinogen, plasma vWf, and the urinary albumin-creatinine ratio (ACR). We were especially interested in whether improvement in glycaemic control was associated with improvement in these cardiovascular risk factors and indicators. Because the diabetic state is thought to be associated with an adverse cardiovascular risk profile, in part through the effects of hyperglycaemia (and insulin resistance), we reasoned that improvement of glycaemic control might be associated with favourable changes of cardiovascular risk factors and indicators. On the other hand, lack of any such associations might to some extent explain the inconsistent effects of improvement of glycaemic control on risk of atherothrombotic disease.<sup>13,14</sup>

## MATERIALS AND METHODS

### Design and measurements

Participants were treated by their own general practitioner (GP). A single physician (FEEvdD) and a diabetes educator performed three-monthly surveillance of treatment-related parameters at the study centre. Results were sent to the GP. The GP (and the patient) then decided whether a subsequent treatment step according to a standard step-up therapy regimen should be taken. Enrolment to the trial was between June 1992 and December 1993, and the trial ended in December 1995. The Ethical Review Committee of the Free University Medical Centre approved the study protocol. The study was approved and performed before results of the UKPDS were reported.

The regimen was a slightly modified version of the practice guidelines for type 2 diabetes of the Netherlands College of General Practitioners.<sup>27</sup> An additional feature of the regimen was a stepwise protocol for initiation of insulin therapy by the GP. The regimen had the usual build-up: tablets in increasing doses up to their usual maximum before other blood-glucose-lowering agents were added. In patients with a body mass index  $\geq 27$  kg/m<sup>2</sup>, metformin was the first step. If the assigned target values for glycaemic control were not reached, a sulphonylurea (SU) – either glibenclamide, gliclazide or glipizide – was added. In patients with a body mass index <27 kg/m<sup>2</sup> SU was the first step. If the assigned target values were not reached on tablets alone, bedtime intermediate-acting insulin was added (and metformin, if any, discontinued). If target values were not reached with this combination therapy, SU was discontinued and twice-daily injections of a mixture of short- and intermediate-acting insulin were started. If glycaemic control remained poor, multiple insulin injection therapy was considered.

Patients were randomly allocated to one of two groups, which differed only in target values for fasting capillary glucose levels. In 'group 6', fasting target values for capillary blood glucose were near-normal glycaemia (<6.5 mmol/l). In the other arm ('group 8'), the fasting treatment target was <8.5 mmol/l, a value considered to be 'acceptable'.<sup>27</sup> Participating GPs were instructed to refrain from any further steps that might lower blood glucose as long as glucose levels were below the allocated target values. Further details of the study population have been described in detail elsewhere.<sup>28</sup> Briefly, 372 Caucasian subjects between 40 and 75 years, were invited. After exclusion of subjects with comorbidity and those who were probably nondiabetic, 232 gave informed consent and participated in the study. Three-monthly assessments included levels of glucose, HbA<sub>1c</sub> and lipids, treatment modality, body mass index, blood pressure and early morning ACR. Six-monthly assessments included serum creatinine, proinsulin, insulin

and fibrinogen. Vwf was measured at baseline, at one and at two years. Systolic and diastolic (Korotkoff V) blood pressure were measured on the right arm of the seated patient with a Hawksley random zero sphygmomanometer.

### Laboratory assessments

All blood samples were taken in the fasting state. Serum and plasma were stored at -20°C for assessment of proinsulin, insulin and vWf, which took place after closure of the data collection. All other assessments were performed on the same day. Venous glucose was measured in sodium fluoride plasma by the glucose dehydrogenase method (Merck, Germany). HbA<sub>1c</sub> was determined in EDTA plasma by ion exchange HPLC (reference range: 4.3 to 6.1%; Modular Diabetes Monitoring System, BioRad, the Netherlands). Immunospecific insulin and proinsulin were measured in serum by double-antibody radioimmunoassays (lot SP21, Linco Research, St Louis, USA for insulin, and Lilly Laboratory for Clinical Research, Indianapolis, USA for proinsulin).<sup>29</sup> Serum total cholesterol, HDL cholesterol and triglycerides were determined by enzymatic colorimetric techniques (CHOD-PAP and CPO-PAP, Boehringer Mannheim, Germany). LDL cholesterol was calculated with the Friedewald formula (not for patients with TG >8.0 mmol/l).<sup>30</sup> Fibrinogen was determined in citrated plasma by a spectrophotometric prothrombin time-derived method (ACL 1000, Instrumentation Laboratory, the Netherlands). Vwf was determined in heparinised plasma by an ELISA and expressed as a percentage of normal pooled plasma (reference range: 50 to 150%).<sup>18,31,32</sup> Urinary and serum creatinine levels were measured by a modified Jaffé method (Boehringer Mannheim, Germany). Urinary albumin was measured by an immunonephelometric method (sensitivity limit: 6.2 mg/l; Beckman, Ireland). For calculation of urinary ACR, we only used early morning samples negative to dipstick tests for nitrite and leucocytes (86% of all samples).

### Statistical analysis

We studied the effects of the random assignment by comparison of groups 6 and 8 at the last available measurements, using t tests,  $\chi^2$  tests or Mann-Whitney U tests. Comparison of baseline and follow-up measurements were carried out using paired t tests, McNemar's  $\chi^2$  tests or Wilcoxon's signed-rank tests. Since the interventions did not yield statistically significant differences between the treatment arms we reanalysed the data focusing on within-subject changes in cardiovascular risk factors and indicators in the entire cohort. Cohort analysis of a randomised clinical trial (RCT) is legitimate, because an RCT is a cohort, with intervention as a determinant. Adjusting the analysis for the intervention (high versus low target) eliminates possible bias caused by this determinant.<sup>33</sup> The cardiovascular risk factors and indicators

considered were proinsulin, insulin, lipids, blood pressure, fibrinogen, vWf and ACR. Normoalbuminuria was distinguished from microalbuminuria and albuminuria using 3.5 mg/mmol as the cut-off.<sup>34</sup>

To study within-subject changes, change rates (slopes) were calculated for each patient separately by linear regression analysis based on all the available measurements.

Preliminary analyses showed that patients with less than the full two years of follow-up were likely to confound analyses. Therefore, we chose to confine all analyses using these change rates to those patients who completed two years of follow-up (n=166). Relations between change rates of glycaemic parameters and change rates of outcome measures were studied by univariate scatter diagrams, and by partial correlations after adjustment for sex, diabetes duration, and group 6 or 8. We also addressed these associations by using analysis of variance (ANOVA) and trend analysis, comparing the change rates of the outcome measures across tertiles of HbA<sub>1c</sub> change rates. All multivariate analyses were performed with and without additional adjustment for change in body weight.

## RESULTS

During the first year, ten patients found participation too much of a burden, six moved and one died (7%). One outlier (a woman with a BMI of 59) was excluded from the analyses. Thus, 106 patients in group 6 and 108 patients in group 8 were included in the analyses. During the second year, 12 of these remaining 214 patients found further participation too burdensome and dropped out, eight were lost to follow-up and two died. At closure of the data collection, 26 patients had not yet reached the two-year visit. Mean duration of follow-up at the time of the analysis was 22 months. Table 1 shows the characteristics of the study population. Fasting plasma glucose was significantly lower in group 6 as compared with group 8, but no clinically meaningful contrast in HbA<sub>1c</sub> had been achieved at a mean follow-up of 22 months. Furthermore, the difference in treatment intensity did not yield statistically significant differences between groups 6 and 8 at follow-up in any of the other outcome measures. In the total population, glycaemic control improved slightly from baseline to follow-up, as reflected by lowering of the fasting glucose levels and lower variability of mean HbA<sub>1c</sub>. Furthermore, total and LDL cholesterol, and blood pressure decreased. In contrast, body mass index, fibrinogen and ACR (increase), and HDL cholesterol (decrease) deteriorated during follow-up. Fasting serum insulin increased significantly due to 23 patients starting insulin injections during follow-up. At baseline 16.5% of the participants were on antihypertensive medication and 13.5% lipid-lowering medication, versus 24.1 and 16.7% at follow-up.

**Table 1**  
*Characteristics at baseline and at follow-up in group 6 (n=106), group 8 (n=108) and the total population (n=214)*

	AT BASELINE		AT FOLLOW-UP*		TOTAL POPULATION	
	GROUP 6	GROUP 8	GROUP 6	GROUP 8	AT BASELINE	AT FOLLOW-UP*
Sex (% male)	53	44			49	
Age (years)	63.3 ± 8.4	63.3 ± 8.3			63.3 ± 8.3	
Diabetes duration	3.4 (0.7-14.2)	3.2 (0.3-12.7)			3.3 (0.5-13.3)	
Cardiovascular history <sup>†</sup>	21	23	26 <sup>c</sup>	29 <sup>c</sup>	22	28 <sup>d</sup>
Hypertension <sup>‡</sup> (% yes)	59	56	59	59	57	59
Smoker (%)	19	22	17	21	20	19
Treatment modality (%)			d	a,d		d
Diet alone	32	37	8	30	35	19
Metformin (M)	4	2	7	5	3	6
Sulphonylurea (SU)	43	38	37	27	40	33
SU + M	8	7	21	14	8	17
Insulin (with or without SU)	14	17	26	25	15	26
Body mass index (kg/m <sup>2</sup> )						
Men	27.9 ± 3.7	28.2 ± 3.7	28.4 ± 3.9 <sup>d</sup>	28.3 ± 3.5	28.0 ± 3.7	28.4 ± 3.7 <sup>c</sup>
Women	28.2 ± 5.9	29.8 ± 4.7	28.5 ± 5.6	30.2 ± 5.3	29.0 ± 5.4	29.4 ± 5.4 <sup>c</sup>
Fasting plasma glucose (mmol/l)	9.4 ± 2.8	9.7 ± 3.3	8.8 ± 2.3	9.5 ± 3.3 <sup>a</sup>	9.6 ± 3.1	9.2 ± 2.9
HbA <sub>1c</sub> (%)	7.2 ± 1.6	7.6 ± 1.9	7.2 ± 1.2	7.4 ± 1.4	7.4 ± 1.7	7.3 ± 1.3
HDL cholesterol (mmol/l)	1.11 ± 0.30	1.12 ± 0.26	1.02 ± 0.30 <sup>d</sup>	1.06 ± 0.27 <sup>d</sup>	1.11 ± 0.28	1.04 ± 0.29 <sup>d</sup>
Triglycerides	2.2 ± 1.5	2.2 ± 1.6	2.2 ± 1.1	2.0 ± 1.1	2.2 ± 1.5	2.1 ± 1.1
Total cholesterol	6.3 ± 1.5	6.4 ± 1.1	5.9 ± 1.2 <sup>d</sup>	6.0 ± 1.0 <sup>d</sup>	6.3 ± 1.3	6.0 ± 1.1 <sup>d</sup>
LDL cholesterol	4.1 ± 1.1	4.2 ± 0.9	3.9 ± 1.0 <sup>d</sup>	4.0 ± 0.9 <sup>d</sup>	4.2 ± 1.0	4.0 ± 1.0 <sup>d</sup>
Systolic blood pressure (mmHg)	152 ± 25	149 ± 22	149 ± 23	145 ± 24 <sup>c</sup>	150 ± 23	147 ± 23 <sup>c</sup>
Diastolic blood pressure	84 ± 12	85 ± 12	83 ± 11	83 ± 12	84 ± 12	83 ± 12 <sup>c</sup>
Insulin (pmol/l) (n=143)	71 (31-158)	67 (43-146)	79 (35-216) <sup>c</sup>	84 (43-200) <sup>c</sup>	69 (38-158)	81 (37-212) <sup>d</sup>
Pro-insulin (n=143)	3.9 (1.2-9.1)	4.8 (1.0-10.6)	4.1 (1.1-9.3)	4.5 (1.2-9.8)	4.3 (1.1-10.4)	4.4 (1.2-9.4)
Fibrinogen (g/l) (n=179)	3.5 ± 0.8	3.6 ± 0.8	3.9 ± 0.9 <sup>d</sup>	3.9 ± 0.9 <sup>d</sup>	3.5 ± 0.8	3.9 ± 0.9 <sup>d</sup>
Von Willebrand factor (%)	128 ± 53	118 ± 48	123 ± 46	122 ± 52	123 ± 51	123 ± 49
Albumin-creatinine ratio (mg/mmol)						
Baseline <3.5 (n=174)	0.8 (0.4-1.8)	0.8 (0.4-2.0)	0.8 (0.4-3.0)	0.8 (0.3-2.6)	0.8 (0.4-1.9)	0.8 (0.4-2.6) <sup>c</sup>
Baseline ≥3.5 (n=40)	8.3 (3.7-137)	10.5 (4.4-73)	9.1 (0.6-89)	7.3 (1.6-74)	9.4 (3.8-73)	8.1 (0.9-74)
Serum creatinine (μmol/l)						
ACR at baseline <3.5	85 ± 17	82 ± 13	89 ± 17 <sup>c</sup>	85 ± 13 <sup>c</sup>	84 ± 15	87 ± 15 <sup>d</sup>
ACR at baseline ≥3.5	87 ± 16	87 ± 16	95 ± 24 <sup>c</sup>	94 ± 19 <sup>d</sup>	87 ± 16	94 ± 22 <sup>d</sup>

Data are percentages, means ± standard deviations, or medians (10<sup>th</sup>-90<sup>th</sup> centile). HbA<sub>1c</sub> = glycated haemoglobin, ACR = albumin creatinine ratio. \* Mean duration of follow-up: 22 months, <sup>†</sup> cardiovascular history is defined as at least one of the following: myocardial infarction, angina pectoris, stroke, transient ischaemic attack, and intermittent claudication, <sup>‡</sup> hypertension is defined as a systolic blood pressure >165 mmHg, and/or a diastolic blood pressure >90 mmHg and/or blood pressure-lowering medication. <sup>a</sup> Group 6 versus group 8, *p* < 0.05, <sup>c</sup> follow-up versus baseline, *p* < 0.05, <sup>d</sup> follow-up versus baseline, *p* < 0.01.

We subsequently analysed changes in the outcome measures in patients who had completed two years of follow-up (n=166) by tertiles of change rates of HbA<sub>1c</sub> (table 2). Results for tertiles of fasting glucose change rates were similar (data not shown). Triglycerides (figure 1), total cholesterol and blood pressure changed most favourably in the tertile with the HbA<sub>1c</sub> decrease, as compared with the other two tertiles. These associations could not be ascribed to the prescription of more lipid-lowering agents or antihypertensives in that tertile (data not shown). Only in patients with an ACR <3.5 mg/mmol at baseline did

changes in ACR show a significant favourable association with HbA<sub>1c</sub> change rates. Changes in vWf were minor and not related to HbA<sub>1c</sub> change rates. Overall, fibrinogen increased, but mainly in the lowest and middle tertiles of HbA<sub>1c</sub> change (table 2 and figure 2). We checked whether adjustment for weight change altered any of the relations under study. This was not the case (data not shown). As shown in tables 1 and 2, there were relatively large mean changes in fibrinogen, which could not be explained by changes in glycaemic control (table 2), or by changes in the prevalence of smokers (data not shown). Upon further

Table 2

Median baseline  $HbA_{1c}$  and adjusted change rates of serum lipids, blood pressure, serum pro-insulin and insulin, plasma fibrinogen and von Willebrand factor, and urinary albumin-creatinine ratio, according to tertiles of  $HbA_{1c}$  change rate during follow-up ( $n=166$ , two-year follow-up)

TERTILES OF $HbA_{1c}$ CHANGE RATE (%/YEAR)	N	MEDIAN BASE- LINE $HbA_{1c}$ (%) (10 <sup>TH</sup> -90 <sup>TH</sup> CENTILE)	MEAN CHANGE RATES (/YEAR) OF										ACR* (MG/MMOL)	
			HDL (mM)	TG (mM)	TC (mM)	LDL (mM)	SBP (mm Hg)	DBP (mm Hg)	INSU- LIN† (pM)	PRO- I (pM)	FIBRIN- OGEN (g/l)	VWF (%)	BASE- LINE <3.5	BASE- LINE ≥3.5
-0.56 (-1.29- -0.19): decrease	55	8.3 (6.7-10.8)	-0.034	-0.17	-0.27	-0.14	-2.6	-0.9	6.3	0.13	0.22	-3.6	-0.12	-0.58
0.11 (-0.08-0.26): stable	56	6.4 (5.5-9.1)	-0.054	0.00	-0.11	-0.05	0.2	-0.8	4.8	-0.10	0.26	1.4	0.13	5.09
0.53 (0.29-0.99): increase	55	6.3 (5.5-8.3)	-0.044	0.08	-0.08	-0.05	1.1	1.5	0.3	0.73	0.15	0.3	0.51	0.96
		P trend	NS	0.00	0.01	NS	NS	0.04	NS	NS	NS	NS	0.00	NS
		P ANOVA	NS	0.01	0.02	NS	0.03	NS	NS	NS	NS	NS	0.01	NS

All change rates of the outcome measures are adjusted for sex, diabetes duration, and randomisation group. Additional adjustment for change in body weight did not make substantial differences in any of the analyses.  $HbA_{1c}$  = glycated haemoglobin, HDL = HDL cholesterol, TG = triglycerides, TC = total cholesterol, LDL = LDL cholesterol, SBP/DBP = systolic/diastolic blood pressure, I = insulin, vWf = von Willebrand factor, ACR = albumin creatinine ratio, NS = not significant ( $p > 0.05$ ). \* Subdivided into ACR <3.5 ( $n=133$ ) and  $\geq 3.5$  mg/mmol ( $n=33$ ) at baseline, † patients on insulin therapy at baseline and (or) at follow-up were excluded ( $n$  of those included: 32/46/38).

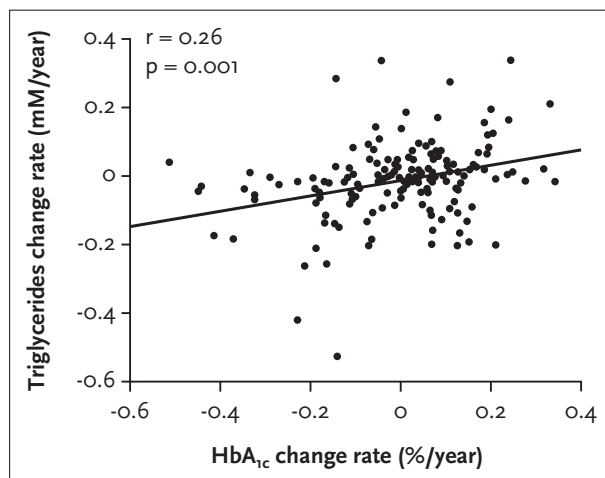


Figure 1  
Scatterplot of the change rate of  $HbA_{1c}$  and the change rate of triglycerides

$r$  = Pearson's correlation coefficient,  $p$  =  $p$  value corresponding to Pearson's correlation coefficient.

analysis, there were significant baseline differences in fibrinogen levels among patients on diet treatment, those on tablets, and those using insulin, which remained when adjusted for age, sex and diabetes duration. These differences were still present at follow-up. Patients using insulin had significantly higher fibrinogen levels than those on diet, while those on tablets had intermediate fibrinogen levels. From baseline to follow-up, fibrinogen increased from  $3.7 \pm 0.9$  to  $4.3 \pm 0.9$  g/l (mean  $\pm$  SD,  $p=0.03$ ) in patients who started insulin treatment ( $n=23$ ), and from  $3.3 \pm 0.6$  to  $3.8 \pm 0.9$  g/l ( $p<0.001$ ) in those

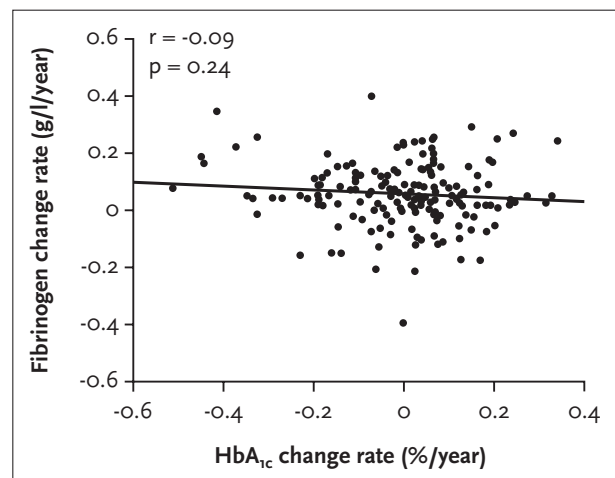


Figure 2  
Scatterplot of the change rate of  $HbA_{1c}$  and the change rate of fibrinogen

$r$  = Pearson's correlation coefficient,  $p$  =  $p$  value corresponding to the Pearson's correlation coefficient.

who changed from diet alone to tablet treatment ( $n=35$ ). In the subgroup who retained baseline treatment throughout the study ( $n=93$ ), fibrinogen increased from  $3.5 \pm 0.8$  to  $3.8 \pm 0.9$  g/l ( $p=0.003$ ). In contrast, in the subgroup who stayed on diet treatment alone throughout the study ( $n=37$ ), fibrinogen remained unchanged ( $3.4 \pm 0.7$  g/l), despite significant worsening of glycaemic control. Among individuals who started insulin treatment at some point during the observation period, the change in fibrinogen levels (measured at 0, 6, 12, 18 and 24 months) was about twice as large in the six-month interval in which the



insulin was started (0.28 g/l) as in the six-month intervals preceding and following the interval in which insulin was started (0.14 g/l). For comparison, plasma glucose continued to decrease after starting insulin, body mass index continued to increase, triglycerides decreased and ACR decreased. The vWf levels during the one-year interval with and without the initiation of insulin treatment showed a slight increase (7.2%) and a slight decrease (-5.2%), respectively. Changes in HDL cholesterol were not clearly associated with any specific treatment step.

The duration of storage of serum and plasma samples (range: 18 to 48 months) did not significantly correlate with the measured levels of insulin, proinsulin and vWf (correlation coefficients: .01, .09, and .07, respectively). This suggests that the quality of the blood samples did not deteriorate significantly during storage at -20°C.

## DISCUSSION

No differences between the two intervention arms were found, except for a slightly lower fasting plasma glucose level. The lack of a greater contrast in fasting glucose levels, and in HbA<sub>1c</sub>, may have been due to two obstacles. Firstly, the eligibility criteria employed may not have been sufficiently restrictive. Secondly, compliance with the study protocol may not have been sufficient to achieve the desired divergence between the treatment arms. Therefore, we reanalysed our data and focused on the question whether different levels of success in lowering the main parameter of antihyperglycaemic treatment, HbA<sub>1c</sub>, would be accompanied by different changes in various cardiovascular risk factors and indicators. As expected, we found that triglycerides, total cholesterol, blood pressure and ACR (only when <3.5 mg/mmol at baseline) exhibited significantly more favourable changes in the tertile with decreasing HbA<sub>1c</sub>, compared with the other tertiles. In contrast, these favourable changes were not found for proinsulin, insulin, fibrinogen and vWf (table 2). For fibrinogen and vWf, this is in accordance with earlier studies.<sup>35-37</sup> Moreover, in the whole group, fibrinogen even showed a significant increase. In conclusion, improvement of glycaemic control – with the strategy employed, i.e. a conventional step-up regime consisting of metformin or sulphonylurea in increasing dosages and insulin if the assigned target values were not reached with metformin or sulphonylurea – was associated with concurrent favourable changes in some, but not all, the cardiovascular risk factors and indicators.

Our results concerning the favourable association of HbA<sub>1c</sub> with ACR in those subjects without microalbuminuria and the absence of such an association in those with microalbuminuria are in accordance with findings in type 1 diabetes that intensified glycaemic control may not have

favourable effects on the progression of the urinary albumin excretion rate once microalbuminuria exists.<sup>38</sup> In addition, Levin *et al.* reported that creatinine clearance among subjects with microalbuminuria deteriorated more rapidly than among subjects without microalbuminuria, regardless of intensity of glycaemic treatment.<sup>39</sup>

Glycaemic changes were not significantly associated with changes in fibrinogen. However, fibrinogen increased after the initiation of insulin treatment (or after an increase of pharmacological treatment in general). Adjustment for diabetes duration, age and sex did not substantially affect differences between those who started insulin and the rest of the population (data not shown). Furthermore, fibrinogen showed a gradual overall increase during the study. However, the yearly change rate was far greater than the relationship of fibrinogen with age in nondiabetics would suggest (except in the subgroup who remained on diet treatment alone throughout the study). In a cross-sectional study, De Boever *et al.* found a 0.14 g/l increase per ten years in a nondiabetic population aged between 35 and 59 years.<sup>40</sup> These findings may be of particular interest. Firstly, considerable variations in cardiovascular event rates could be attributable to relatively small variations in fibrinogen levels. Meade *et al.* have shown that a fibrinogen level elevation of 0.6 g/l was associated with an 84% increased risk of stroke within the next five years.<sup>41</sup> In addition, Danesh *et al.* showed, in a meta-analysis of prospective studies, an 80% increased risk for coronary heart disease for each increment in fibrinogen of 1.0 g/l.<sup>42</sup> Secondly, the VA CSDM feasibility trial reported an unexpectedly high incidence of cardiovascular events in a group of male type 2 diabetic patients who started insulin treatment.<sup>14</sup> Fibrinogen level rose significantly in the first year and returned to baseline at two years.<sup>43</sup>

Insulin and proinsulin have been recognised as potentially atherogenic substances, although it is not clear whether insulin by injection has effects comparable with those seen in hyperinsulinaemia related to insulin resistance.<sup>44,45</sup> A study in the offspring of hypertensive men demonstrated that fibrinogen is closely related to plasma insulin and most components of the insulin resistance syndrome.<sup>46</sup> In contrast De Feo *et al.* demonstrated that induced insulin deficiency was associated with an increase in fibrinogen, suggesting an acute phase protein response.<sup>47</sup> *In vitro* experiments lend support to possible adverse effects of proinsulin and insulin on fibrinolysis, acting via both liver and endothelium.<sup>48</sup> The present findings could be in accordance with an effect of treatment intensification on the production of fibrinogen by hepatocytes *in vivo*. *In vitro* studies, however, have shown inconsistent results in this respect. Incubation of hepatocytes with insulin for less than 24 hours had no influence on fibrinogen synthesis,<sup>49,50</sup> while studies with longer incubation showed no effect,<sup>51</sup> decreased<sup>52</sup> or increased fibrinogen synthesis.<sup>53</sup> Moreover,

in our study fibrinogen change rate was not related to insulin and proinsulin change rates (data not shown). In sum, our data do not provide an explanation for the fibrinogen changes that were observed. In conclusion, our study suggests that further improvement of glycaemic control in moderately well-controlled type 2 diabetes is not associated with concurrent favourable changes in some cardiovascular risk indicators that are typically associated with type 2 diabetes. We stress that our findings on glycaemic control cannot be distinguished from the therapeutic strategy employed, i.e. a conventional step-up regime consisting of metformin or sulphonylurea in increasing dosages and insulin if the assigned target values were not reached. Therefore, we cannot exclude that improvement of glycaemic control through other strategies may yield better results in terms of cardiovascular risk factor amelioration. Nevertheless, our findings may provide a framework for understanding the disappointing effects of intensified glycaemic control on risk of macrovascular disease. Our finding that fibrinogen levels may increase after starting insulin treatment of particular concern and warrants further study.

## REFERENCES

- Kannel WB, D'Agostino RB, Wilson PWF, Belanger AJ, Gagnon DR. Diabetes, fibrinogen, and risk of cardiovascular disease: the Framingham experience. *Am Heart J* 1990;120:672-6.
- Nathan DM, Meigs J, Singer DE. The epidemiology of cardiovascular disease in type 2 diabetes mellitus: how sweet it is ... or is it? *Lancet* 1997;350(suppl 1):S4-9.
- Wang PH, Lau J, Chalmers TC. Meta-analysis of effects of intensive blood-glucose control on late complications of type I diabetes. *Lancet* 1993;341:1306-9.
- The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993;329:977-86.
- Gilbert RE, Tsalamandris C, Bach LA, et al. Long-term glycaemic control and the rate of progression of early diabetic kidney disease. *Kidney International* 1993;44:885-9.
- Ohkubo Y, Kishikawa H, Araki E, et al. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract* 1995;28:103-17.
- Uusitupa MJ, Niskanen LK, Siitonen O, Voutilainen E, Pyörälä K. Ten-year cardiovascular mortality in relation to risk factors and abnormalities in lipoprotein composition in type 2 (non-insulin-dependent) diabetic and non-diabetic subjects. *Diabetologia* 1993;36:1175-84.
- Kuusisto J, Mykkanen L, Pyörälä K, Laakso M. NIDDM and its metabolic control predict coronary heart disease in elderly subjects. *Diabetes* 1994;43:960-7.
- Moss SE, Klein R, Klein BEK, Meuer SM. The association of glycemia and cause-specific mortality in a diabetic population. *Arch Intern Med* 1994;154:2473-9.
- Lehto S, Ronnemaa T, Haffner SM, Pyörälä K, Kallio V, Laakso M. Dyslipidemia and hyperglycemia predict coronary heart disease events in middle-aged patients with NIDDM. *Diabetes* 1997;46:1354-9.
- Groeneveld Y, Petri H, Hermans J, Springer MP. Relationship between blood glucose level and mortality in type 2 diabetes mellitus: a systematic review. *Diabet Med* 1999;16:2-13.
- Vegt F, de Dekker JM, Ruhe HG, et al. Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: the Hoorn Study. *Diabetologia* 1999;42:926-31.
- UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998;352:837-53.
- Abraira C, Colwell J, Nuttall F, et al. Cardiovascular events and correlates in the Veterans Affairs Diabetes Feasibility Trial. Veterans Affairs Cooperative Study on Glycemic Control and Complications in Type II Diabetes. *Arch Intern Med* 1997;157:181-8.
- Ganda OP, Arkin CF. Hyperfibrinogenemia: an important risk factor for vascular complications in diabetes. *Diabetes Care* 1992;15:1245-50.
- Meigs JB, Mittleman MA, Nathan DM, et al. Hyperinsulinemia, hyperglycemia, and impaired hemostasis: the Framingham Offspring Study. *JAMA* 2000;283:221-8.
- Saito I, Folsom AR, Brancati FL, Duncan BB, Chambless LE, McGovern PG. Nontraditional risk factors for coronary heart disease incidence among persons with diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. *Ann Intern Med* 2000;133:81-91.
- Stehouwer CDA, Nauta JJP, Zeldenrust GC, Hackeng WHL, Donker AJM, Ottolander GJH den. Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non-insulin-dependent diabetes mellitus. *Lancet* 1992;340:319-23.
- Conlan MG, Folsom AR, Finch A, et al. Associations of Factor VII and von Willebrand Factor with age, race, sex, and risk factors for atherosclerosis: the Atherosclerosis Risk in Communities (ARIC) Study. *Thromb Haemostas* 1993;70:380-5.
- Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. *N Engl J Med* 1984;310:356-60.
- Mattock MB, Morrish NJ, Viberti G, Keen H, Fitzgerald AP, Jackson G. Prospective study of microalbuminuria as predictor of mortality in NIDDM. *Diabetes* 1992;41:736-41.
- Beatty OL, Ritchie CM, Bell PM, Hadden DR, Kennedy L, Atkinson AB. Microalbuminuria as identified by a spot morning urine specimen in non-insulin-treated diabetes: an eight-year follow-up study. *Diabet Med* 1995;12:261-6.
- MacLeod JM, Lutale J, Marshall SM. Albumin excretion and vascular deaths in NIDDM. *Diabetologia* 1995;38:610-6.
- Jager A, Hinsbergh VW van, Kostense PJ, et al. Von Willebrand factor, C-reactive protein, and 5-year mortality in diabetic and nondiabetic subjects: the Hoorn Study. *Arterioscler Thromb Vasc Biol* 1999;19:3071-8.
- Jager A, Kostense PJ, Ruhe HG, et al. Microalbuminuria and peripheral arterial disease are independent predictors of cardiovascular and all-cause mortality, especially among hypertensive subjects: five-year follow-up of the Hoorn Study. *Arterioscler Thromb Vasc Biol* 1999;19:617-24.



26. Jager A, Hinsbergh VW van, Kostense PJ, et al. Prognostic implications of retinopathy and a high plasma von Willebrand factor concentration in type 2 diabetic subjects with microalbuminuria. *Nephrol Dial Transplant* 2001;16:529-36.
27. Cromme PVM, Mulder JD, Rutten GEHM, Zuidweg J, Thomas S. NHG-Standaard Diabetes mellitus type II. In: Rutten GEHM, Thomas S (eds.). Utrecht: Nederlands Huisartsen Genootschap, 1993.
28. Does FE van der, Neeling JN de, Snoek FJ, et al. Randomized study of two different target levels of glycemic control within the acceptable range in type 2 diabetes. Effects on well-being at 1 year. *Diabetes Care* 1998;21:2085-93.
29. Bowers RR, Wolny JD, Frank BH. A rapid and sensitive radioimmunoassay for the measurement of proinsulin in human serum. *Diabetes* 1992;41:1084-90.
30. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499-502.
31. Ingerslev JA. A sensitive ELISA for von Willebrand factor (vWF:Ag). *Scand J Clin Lab Invest* 1987;47:143-9.
32. Tranquille N, Emeis JJ. The simultaneous acute release of tissue-type plasminogen activator and von Willebrand factor in the perfused rat hindleg region. *Thromb Haemostas* 1990;63:454-8.
33. Poppel MN van, Koes BW, Deville W, Smid T, Bouter LM. Risk factors for back pain incidence in industry: a prospective study. *Pain* 1998;77:81-6.
34. Gatling W, Knight C, Mullee MA, Hill RD. Microalbuminuria in diabetes: a population study of the prevalence and an assessment of three screening tests. *Diabetic Med* 1988;5:343-7.
35. Knöbl P, Scherthaner G, Schnack C, et al. Haemostatic abnormalities persist despite glycaemic improvement by insulin therapy in lean type 2 diabetic patients. *Thrombosis Haemostasis* 1994;71:692-7.
36. Knaap JH v/d, Boer AC de, Pannebakker MAG, Heerde W van, Ottolander GJH den. The effect of diabetes regulation on platelet release, fibrinolysis and coagulation tests, before and after stimulation with DDAVP. *Thromb Haemostas* 1985;53:118-21.
37. Yudkin JS, Panahloo A, Stehouwer C, et al. The influence of improved glycaemic control with insulin and sulphonylureas on acute phase and endothelial markers in Type II diabetic subjects. *Diabetologia* 2000;43:1099-106.
38. Diabetes Control and Complications (DCCT) Research Group. Effect of intensive therapy on the development and progression of diabetic nephropathy in the diabetes control and complications trial. *Kidney Int* 1995;47:1703-20.
39. Levin SR, Coburn JW, Abaira C, et al. Effect of intensive glycemic control on microalbuminuria in type 2 diabetes. Veterans Affairs Cooperative Study on Glycemic Control and Complications in Type 2 Diabetes Feasibility Trial Investigators. *Diabetes Care* 2000;23:1478-85.
40. Boever E de, Bacquer D de, Braeckman L, Baele G, Rosseneu M, Backer G de. Relation of fibrinogen to lifestyles and to cardiovascular risk factors in a working population. *Int J Epidemiol* 1995;24:915-21.
41. Meade TW, Mellows S, Brozovic M, et al. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. *Lancet* 1986;2:533-7.
42. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *JAMA* 1998;279:1477-82.
43. Emanuele N, Azad N, Abaira C, et al. Effect of intensive glycemic control on fibrinogen, lipids, and lipoproteins: Veterans Affairs Cooperative Study in Type II Diabetes Mellitus. *Arch Intern Med* 1998;158:2485-90.
44. Stern MP. Do non-insulin-dependent diabetes mellitus and cardiovascular disease share common antecedents? *Ann Intern Med* 1996;124(1 pt 2):110-6.
45. Ruige JB, Assendelft WJ, Dekker JM, Kostense PJ, Heine RJ, Bouter LM. Insulin and risk of cardiovascular disease: a meta-analysis. *Circulation* 1998;97:996-1001.
46. Valek J, Valkova L, Vlasakova Z, Topinka V. Increased fibrinogen levels in the offspring of hypertensive men. Relation with hyperinsulinemia and the metabolic syndrome. *Arterioscler Thromb Vasc Biol* 1995;15:2229-33.
47. Feo P de, Gaisano MG, Haymond MW. Differential effects of insulin deficiency on albumin and fibrinogen synthesis in humans. *J Clin Invest* 1991;88:833-40.
48. Schneider DJ, Nordt TK, Sobel BE. Attenuated fibrinolysis and accelerated atherogenesis in type II diabetic patients. *Diabetes* 1993;42:1-7.
49. Alessi MC, Juhan-Vague I, Kooistra T, Declercq PJ, Collen D. Insulin stimulates the synthesis of Plasminogen Activator Inhibitor 1 by the human hepatocellular cell line Hep G2. *Thromb Haemostas* 1988;60:491-4.
50. Kooistra T, Bosma PJ, Töns HAM, Berg AP van den, Meyer P, Princen HMG. Plasminogen Activator Inhibitor 1: biosynthesis and mRNA level are increased by insulin in cultured human hepatocytes. *Thromb Haemostas* 1989;62:723-8.
51. Jeejeebhoy KN, Ho J, Greenberg GR, Phillips MJ, Bruce-Robertson A, Sodtke U. Albumin, fibrinogen and transferrin synthesis in isolated rat hepatocyte suspensions: a model for the study of plasma protein synthesis. *Biochem J* 1975;146:141-5.
52. Thompson D, Harrison SP, Evans SW, Whicher JT. Insulin modulation of acute-phase protein production in a human hepatoma cell line. *Cytokine* 1991;3:619-26.
53. Grieninger G, Plant PW, Liang TJ, et al. Hormonal regulation of fibrinogen synthesis in cultured hepatocytes. *Ann NY Acad Sci* 1983;408:469-89.